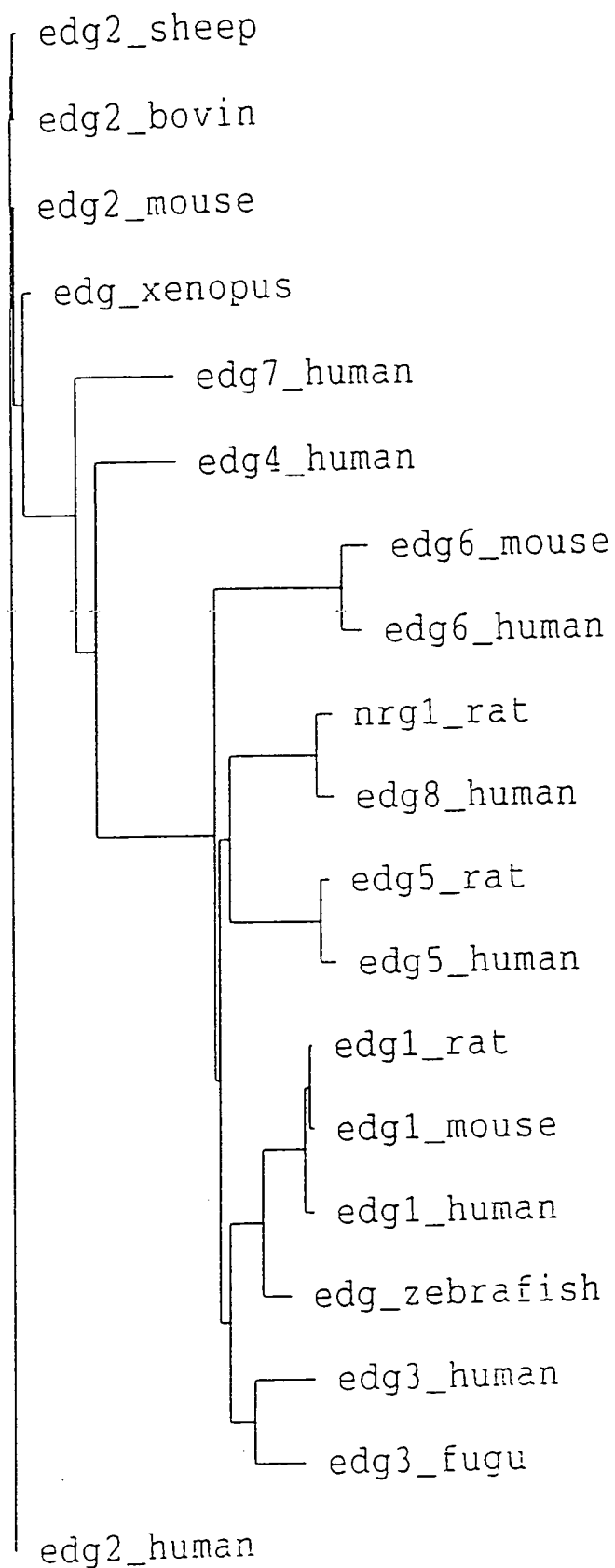FIG 1A:

```
   1 ATGGAGTCGGGGCTGCTGCGGCCGGCGCCGGTGAGCGAGGTCATCGTCCTGCATTACAAC
     M  E  S  G  L  L  R  P  A  P  V  S  E  V  I  V  L  H  Y  N

  61 TACACCGGCAAGCTCCGCGGTGCGCGCTACCAGCCGGGTGCCGGCCTGCGCGCCGACGCC
     Y  T  G  K  L  R  G  A  R  Y  Q  P  G  A  G  L  R  A  D  A

 121 GTGGTGTGCCTGGCGGTGTGCGCCTTCATCGTGCTAGAGAATCTAGCCGTGTTGTTGGTG
     V  V  C  L  A  V  C  A  F  I  V  L  E  N  L  A  V  L  L  V

 181 CTCGGACGCCACCCGCGCTTCCACGCTCCCATGTTCCTGCTCCTGGGCAGCCTCACGTTG
     L  G  R  H  P  R  F  H  A  P  M  F  L  L  L  G  S  L  T  L

 241 TCGGATCTGCTGGCAGGCGCCGCCTACGCCGCCAACATCCTACTGTCGGGGCCGCTCACG
     S  D  L  L  A  G  A  A  Y  A  A  N  I  L  L  S  G  P  L  T

 301 CTGAAACTGTCCCCCGCGCTCTGGTTCGCACGGGAGGGAGGCGTCTTCGTGGCACTCACT
     L  K  L  S  P  A  L  W  F  A  R  E  G  G  V  F  V  A  L  T

 361 GCGTCCGTGCTGAGCCTCCTGGCCATCGCGCTGGAGCGCAGCCTCACCATGGCGCGCAGG
     A  S  V  L  S  L  L  A  I  A  L  E  R  S  L  T  M  A  R  R

 421 GGGCCCGCGCCCGTCTCCAGTCGGGGGCGCACGCTGGCGATGGCAGCCGCGGCCTGGGGC
     G  P  A  P  V  S  S  R  G  R  T  L  A  M  A  A  A  A  W  G

 481 GTGTCGCTGCTCCTCGGGCTCCTGCCAGCGCTGGGCTGGAATTGCCTGGGTCGCCTGGAC
     V  S  L  L  L  G  L  L  P  A  L  G  W  N  C  L  G  R  L  D

 541 GCTTGCTCCACTGTCTTGCCGCTCTACGCCAAGGCCTACGTGCTCTTCTGCGTGCTCGCC
     A  C  S  T  V  L  P  L  Y  A  K  A  Y  V  L  F  C  V  L  A

 601 TTCGTGGGCATCCTGGCCGCTATCTGTGCACTCTACGCGCGCATCTACTGCCAGGTACGC
     F  V  G  I  L  A  A  I  C  A  L  Y  A  R  I  Y  C  Q  V  R

 661 GCCAACGCGCGGCGCCTGCCGGCACGGCCCGGGACTGCGGGGACCACCTCGACCCGGGCG
     A  N  A  R  R  L  P  A  R  P  G  T  A  G  T  T  S  T  R  A

 721 CGTCGCAAGCCGCGCTCGCTGGCCTTGCTGCGCACGCTCAGCGTGGTGCTCCTGGCCTTT
     R  R  K  P  R  S  L  A  L  L  R  T  L  S  V  V  L  L  A  F

 781 GTGGCATGTTGGGGCCCCCTCTTCCTGCTGCTGTTGCTCGACGTGGCGTGCCCGGCGCGC
     V  A  C  W  G  P  L  F  L  L  L  L  L  D  V  A  C  P  A  R

 841 ACCTGTCCTGTACTCCTGCAGGCCGATCCCTTCCTGGGACTGGCCATGGCCAACTCACTT
     T  C  P  V  L  L  Q  A  D  P  F  L  G  L  A  M  A  N  S  L

 901 CTGAACCCCATCATCTACACGCTCACCAACCGCGACCTGCGCCACGCGCTCCTGCGCCTG
     L  N  P  I  I  Y  T  L  T  N  R  D  L  R  H  A  L  L  R  L

 961 GTCTGCTGCGGACGCCACTCCTGCGGCAGAGACCCGAGTGGCTCCCAGCAGTCGGCGAGC
     V  C  C  G  R  H  S  C  G  R  D  P  S  G  S  Q  Q  S  A  S

1021 GCGGCTGAGGCTTCCGGGGGGCCTGCGCCGCTGCCTGCCCCCGGGCCTTGATGGGAGCTTC
     A  A  E  A  S  G  G  L  R  R  C  L  P  P  G  L  D  G  S  F

1081 AGCGGCTCGGAGCGCTCATCGCCCCAGCGCGACGGGCTGGACACCAGCGGCTCCACAGGC
     S  G  S  E  R  S  S  P  Q  R  D  G  L  D  T  S  G  S  T  G

1141 AGCCCCGGTGCACCCACAGCCGCCCGGACTCTGGTATCAGAACCGGCTGCAGACTGA
     S  P  G  A  P  T  A  A  R  T  L  V  S  E  P  A  A  D  *
```

FIG 1B



edg2_sheep

edg2_bovin

edg2_mouse

edg_xenopus

edg7_human

edg4_human

edg6_mouse

edg6_human

nrg1_rat

edg8_human

edg5_rat

edg5_human

edg1_rat

edg1_mouse

edg1_human

edg_zebrafish

edg3_human

edg3_fugu

edg2_human

```
                 1                                                        60
edg2_human   MAAISTSIPV ISQPQFTAMN EPQCFYNESI AFFYNRSGKH LAT.EWNTVS KLVMGL..GI
edg7_human   ---------- --------MN NE..CHYDKHM DFFNIRSNTD TVD.OW.TGT KLVIVLCVGT
edg4_human   ---------- -------MVI NGQCYYNETI GFFYNNSGKE LSS.HWR..P KDVTVVALGL
edg1_human   -----MGPTS VPLVKAHRSS VSDYVNYDII VRHYNYTGKL ..NISADKEN SIKLTSVVFI
edg3_human   ---------- --MATALPPR LQPVRGNETL REHYQYVGKL AGRLKEASEG S.TITTVLFL
edg5_human   ---------- ------MGSL YSEYLNPNKV QEHYNYTKE. ..TLETQETT SRQYASAFIV
edg8_human   ---------- -----MESGL LRPAPVSEVI VLHYNYTGKL RG.ARYQPGA GLRADAVVCL
edg6_human   -----MNATG TPVAPESCQQ LAAGGHSRLI VLHYNHSGRL AGR.GGPEDG GLGALRGLSV

                 61                                                      120
edg2_human   TVCIFIMLAN LLVMVAIYVN RRFHFPIYYL MANLAAADFF AGLAYFYLMF NTGPNTRRLT
edg7_human   FFCLFIFFSN SLVIAAVIKN RKFHFPFYYL LANLAAADFF AGIAYVFLMF NTGPVSKTLT
edg4_human   TVSVLVLLTN LLVIAAIASN RRFHQPIYYL LGNLAAADLF AGVAYLFLMF HTGPRTARLS
edg1_human   LICCFIILEN IFVLLTIWKT KKFHFPMYYL IGNLALSDLL AGVAYTANLL LSGATTYKLT
edg3_human   VICSFIVLEN LMVLIAIWKN NKFHNRMYFF IGNLALCDLL AGIAYKVNIL MSGKKTFSLS
edg5_human   ILCCAIVVEN LLVLIAVARN SKFHSAMYLF LGNLAASDLL AGVAFVANTL LSGSVTLRLT
edg8_human   AVCAFIVLEN LAVLLVLGRH PRFHAPMFLL LGSLTLSDLL AGAAYAANIL LSGPLTLKLS
edg6_human   AASCLVVLEN LLVLAAITSH MRSRFWVYYC LVNITLSDLL TGAAYLANVL LSGARTFRLA

                 121                                                     180
edg2_human   VSTWLLRQGL IDTSLTASVA NLLAIAIERH ITVFR.MQLH TRMSNRRVVV VIVVIWTMAI
edg7_human   VNRWFLRQGL LDSSLTASLT NLLVIAVERH MSIMR.MRVH SNLTKKRVTL LILLVWAIAI
edg4_human   LEGWFLRQGL LDTSLTASVA TLLAIAVERH RSVMA.VQLH SRLPRGRVVM LIVGVWVAAL
edg1_human   PAQWFLREGS MFVALSASVF SLLAIAIERY ITMLK.MKLH NGSNNFRLFL LISACWVISL
edg3_human   PTVWFLREGS MFVALGASTC SLLAIAIERH LTMIK.MRPY DANKRHRVFL LIGMCWLIAF
edg5_human   PVQWFAREGS ASITLSASVF SLLAIAIERH VAIAK.VKLY GSDKSCFMLL LIGASWLISL
edg8_human   PALWFAREGG VFVALTASVL SLLAIALERS LTMAR.RGPA PVSSRGRTLA MAAAAWGVSL
edg6_human   PAQWFLREGL LFTALAASTF SLLFTAGERF ATMVRPVAES GATXTSPVYG FIGLCWLLAA

                 181                                                     240
edg2_human   VMGAIPSVGW NCICDIENCS NMAPLYSDSY LVFWAIFNLV TEVVMVVLYA HIFGYVRQRT
edg7_human   FMGAVPTLGW NCLCNISACS SLAPIYSRSY LVFWTVSNLM AFLIMVVVYL RIYVYVKRKT
edg4_human   GLGLLPAHSW HCLCALDRCS RMAPLLSRSY LAVWALSSLL VFLLMVAVYT RIFFYVRRRV
edg1_human   ILGGLPIMGW NCISALSSCS TVLPLYHKHY ILFCTTVFTL LLLSIVILYC RIYSLVRTRS
edg3_human   TLGALPILGW NCLHNLPCCS TILPLYSKHY IAFCISIFTA ILVTIVILYA RIYFLVRSSH
edg5_human   VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI ILLAIVALYV RIYCVVRSSH
edg8_human   LLGLLPALGW NCLGRLDACS TVLPLYAKHY VLFCVLAFVG ILAAICALYA RIYCQVRANA
edg6_human   LLGMLPLLGW NCLCAFDRCS SLLPLYSKHY ILFCLVIFAG VLATIMGLYG AIFRLVQASG

                 241                                                     300
edg2_human   MRMSRHSSGP R......RNR DTMMSLLKTV VIVLGAFIIC WTPGLVLLLL D.VCCP..QC
edg7_human   NVLSPHTSGS I......SRR RTPMKLMKTV MTVLGAFVVC WTPGLVVLLL DGLNCR..QC
edg4_human   QRMAEHVSCH P......RYR ETTLSLVKTV VIILGAFVVC WTPGQVVLLL DGLGCE..SC
edg1_human   RRLTFR.... .KNISKASRS SENVALLKTV IIVLSVFIAC WAPLFILLLL DV.GCKVKTC
edg3_human   RKVANH.... .NN......S ERSMALLRTV VIVVSVFIAC WSPLFILFLI DV.ACRVQAC
edg5_human   ADMA....... ...A PQTLALLKTV TIVLGVFIVC WLPAFSILLL DY.ACPVHSC
edg8_human   RRLPARPGTA GTTSTRARRK PRSLALLRTL SVVLLAFVAC WGPLFLLLLL DV.ACPARTC
edg6_human   QKAP...... ...RPAARRK ARR..LLKTV LMILLAFLVC WGPLFGLLLA DVFGSNLWAQ

                 301                                                     360
edg2_human   DVLAYEKFFL LLAEFNSAMN PIIYSYRDKE MSATFRQILC CQRSENPTGP TESSDRSASS
edg7_human   GVQHVKRWFL LLALLNSVVN PIIYSYKDED MYGTMKKMIC CFSQENP... ....ERRPSR
edg4_human   NVLAVEKYFL LLAEANSLVN AAVYSCRDAE MRRTFRRLLC CACLRQSTRE SVHYTSSACG
edg1_human   DILFRADYFL VLAVLNSGTN PIIYTLTNKE MRRAFIRIMS CCKCPSGD.. .........S
edg3_human   PILFKAQWFI VLAVLNSVVN PVIYTLASKE MRRAFFRLV. .CNC.LVR.. .........G
edg5_human   PILYKAHYFF AVSTLNSLLN PVIYTWRSRD LRREVLRPLQ CWRPGVGV.. .........Q
edg8_human   PVLLQAQPFL GLAMANSLLN PIIYTLTJRD LRHALLRLVC CGRHSCGRDP SGS..QQSAS
edg6_human   EYLRGMDWIL ALAVLNSAVN PIIYSFRSRE VCRAVLSFLC CGCLRLGMRG PGDCLARAVE

                 361                                                     418
edg2_human   LNHTILAGVH SNDHSVV--- ---------- ---------- ---------- --------
edg7_human   IPSTVLSRSD TGSQYIEDSI SQGAVCNKST S--------- ---------- --------
edg4_human   GASTRIMLPE NGHPLMTPPF SYLELQRYAA SNKSTAPDDL WVLLAQPNCQ Q-------
edg1_human   AGKFKRPIIA GMEFSRSK.. .SDNSSHPQK DEGDNPETIM SSGNVNSSS- --------
edg3_human   RGARASPIQP ALDPSRSKSS SSNNSSHSPK VKEDLPHTGP SSCIMQKNAA LQNGIFCN
edg5_human   GRRVGTPGH HLLPLRSSSS LERGMHMPTS PTFLEGNTVV ---------- --------
edg8_human   AAEASGGLRR CLPPGLDGSF SGSERSSPQR CGLDTSGSTG SPGAPTAART LVSEPAAD
edg6_human   AHSGASTTDS SLRP.RDSFR GSRSLSFRMR EPLSSISSVR SI-------- --------
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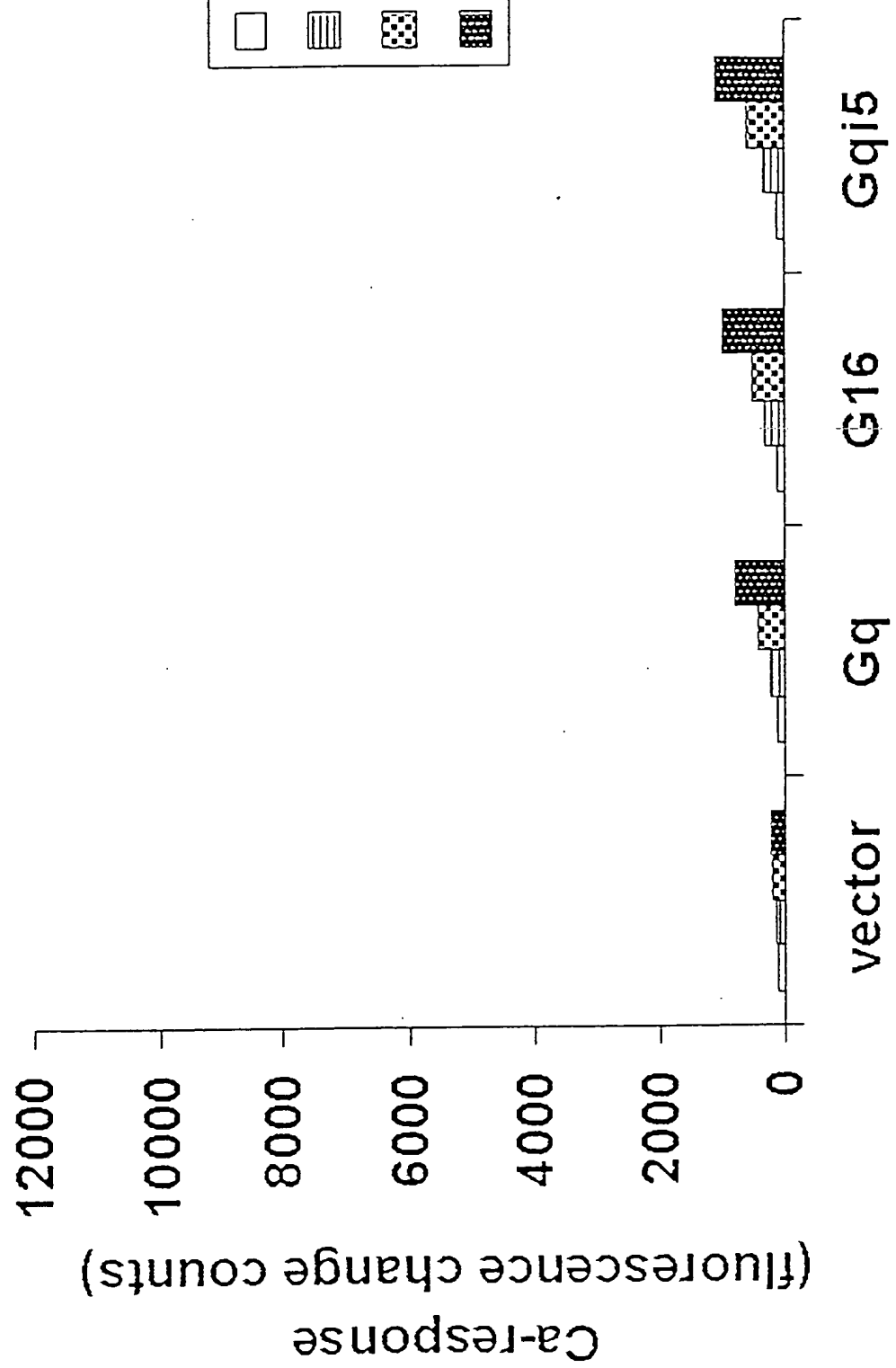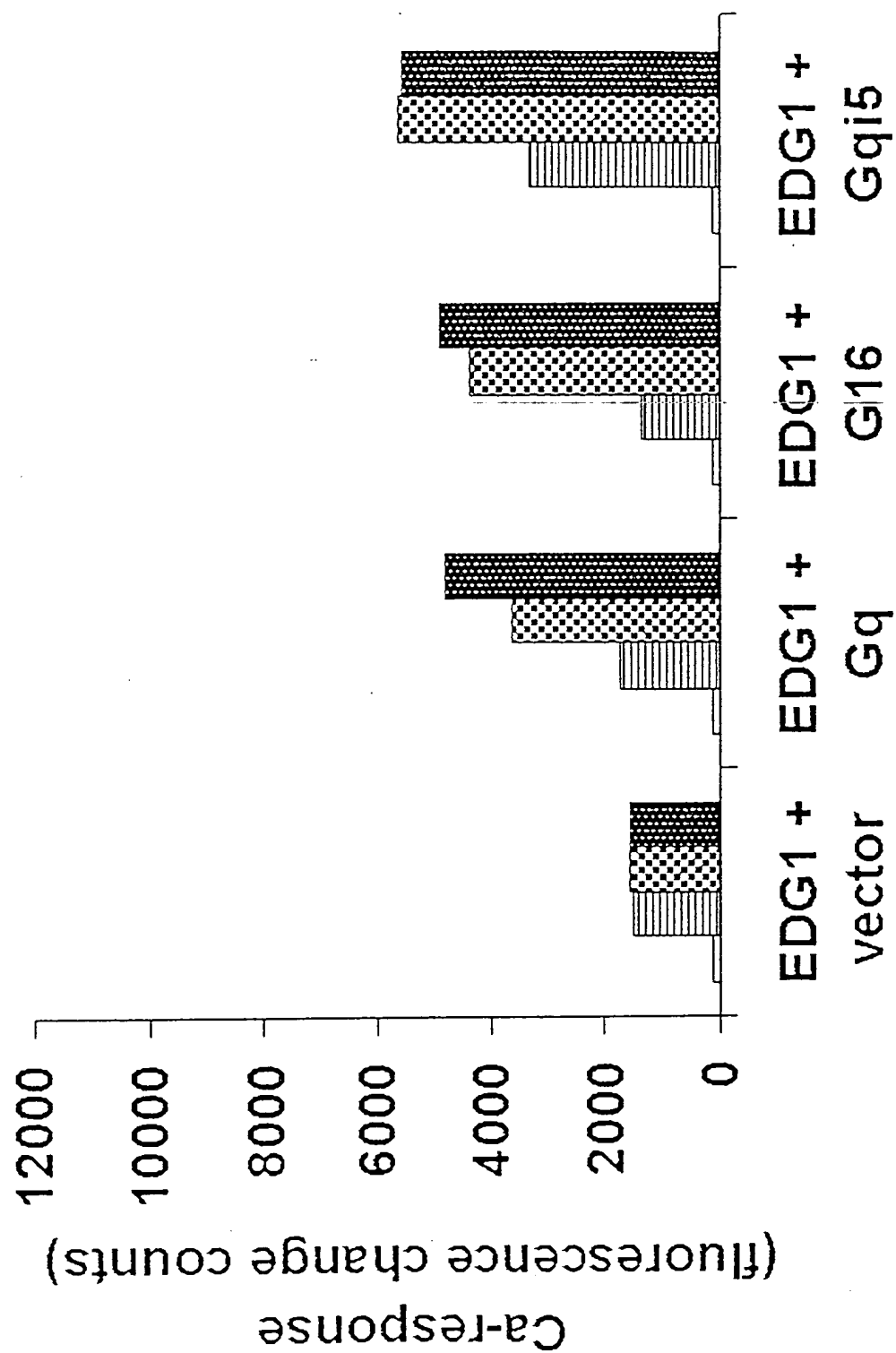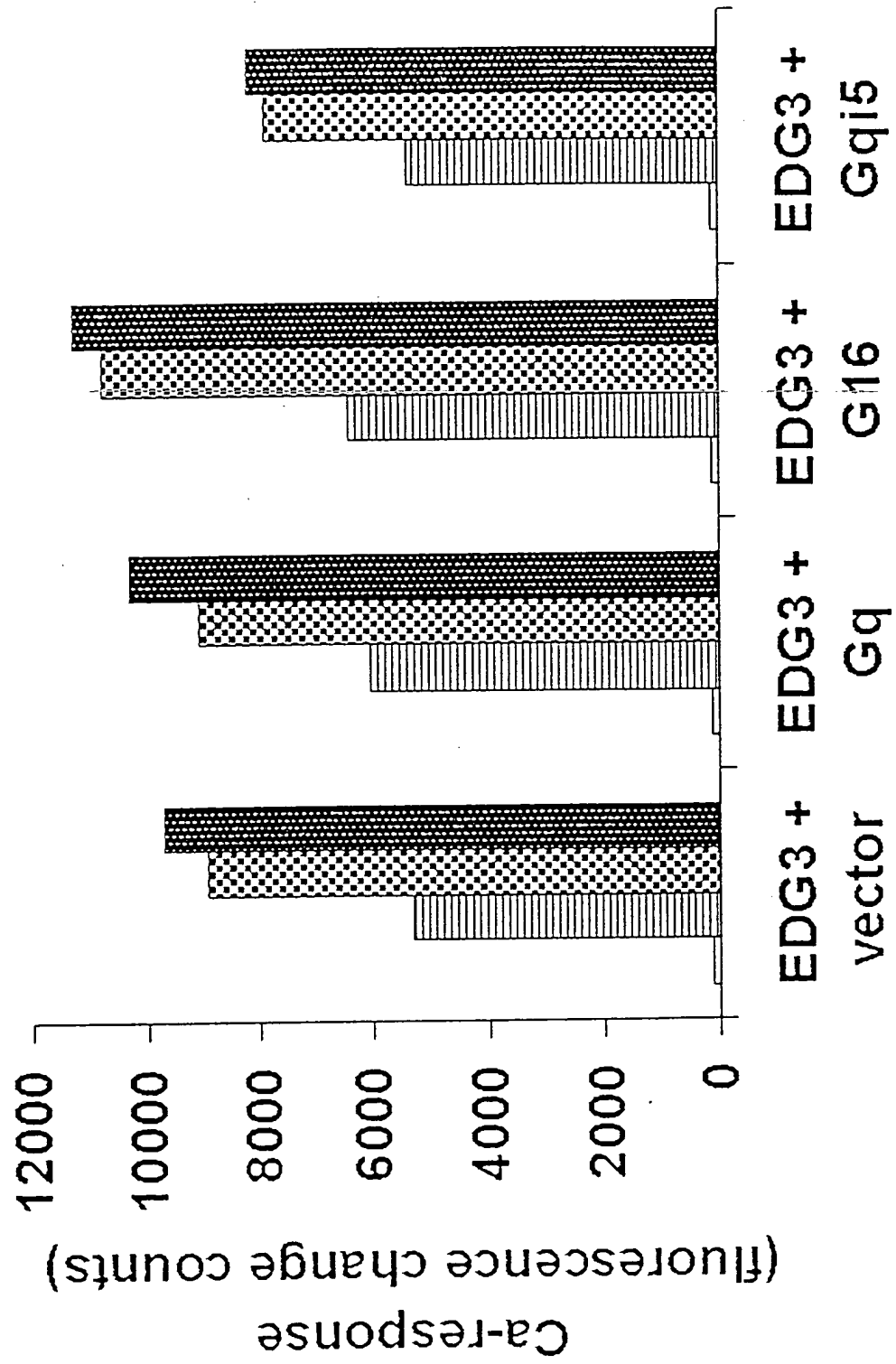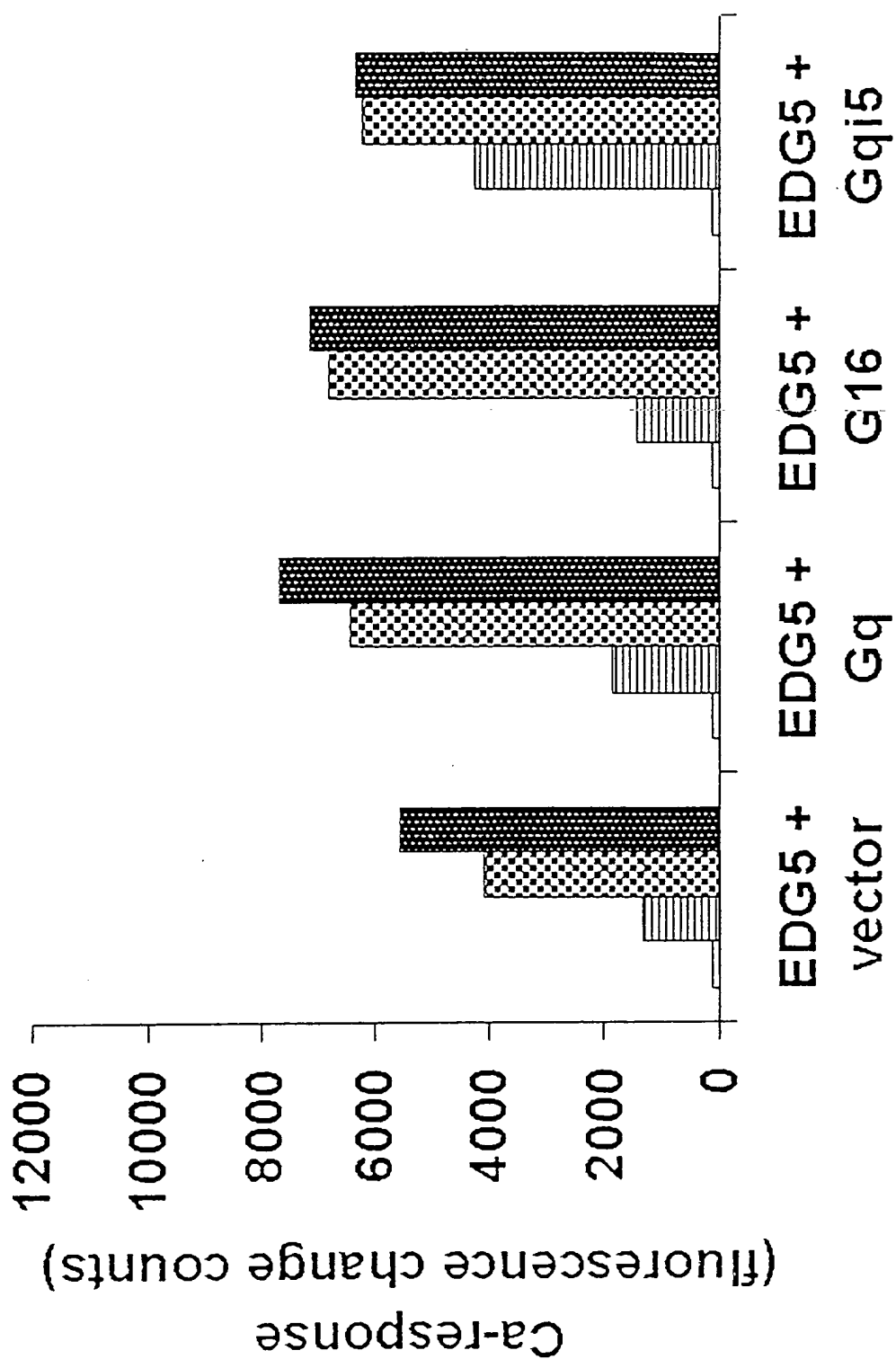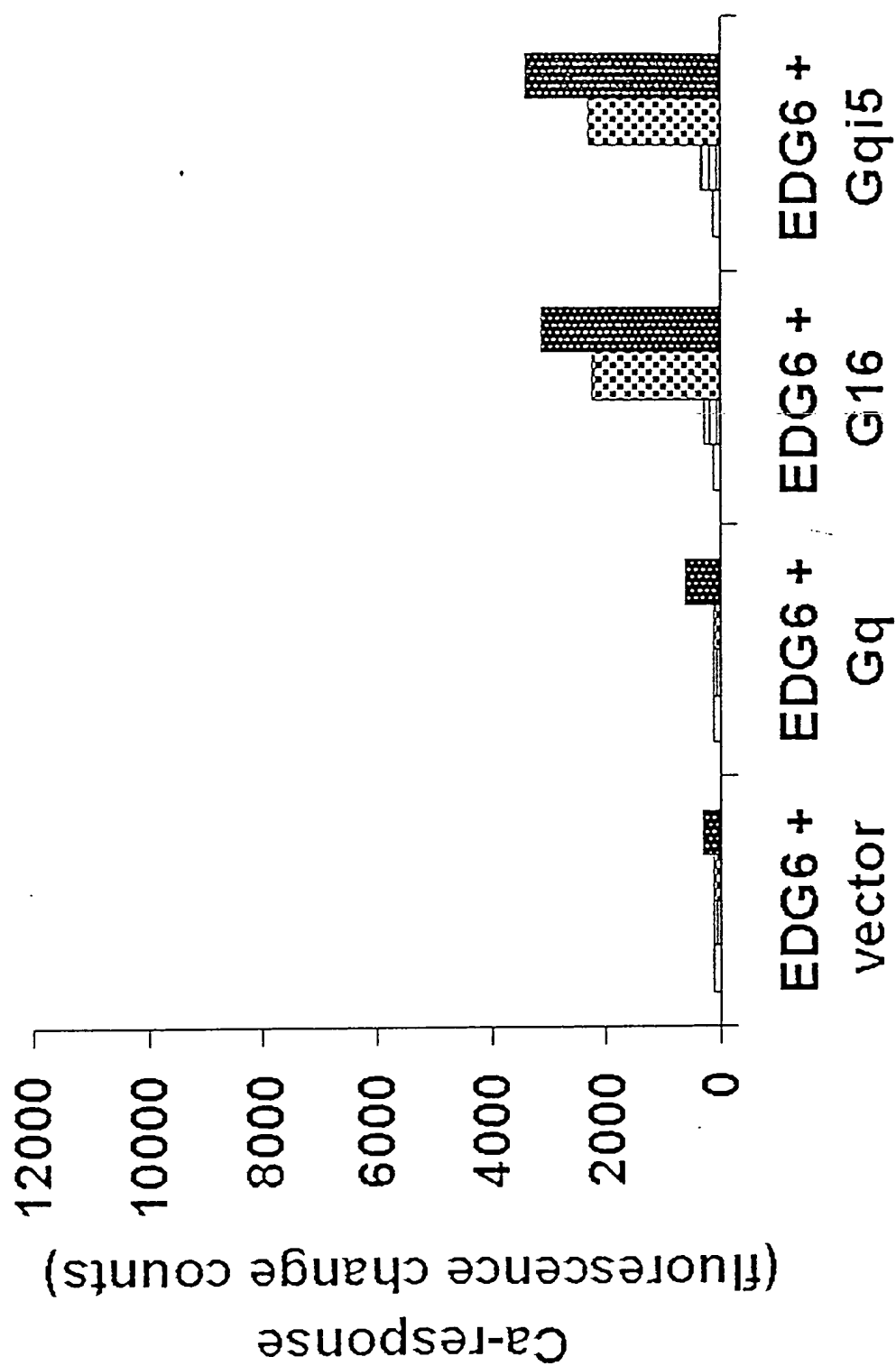
Fig. 1C

FIG 2A



Legend:
- □ 0 nM
- ▥ 10 nM
- ▦ 100 nM
- ▨ 1000 nM

X-axis labels: vector, Gq, G16, Gqi5

Y-axis: Ca-response (fluorescence change counts), 0, 2000, 4000, 6000, 8000, 10000, 12000

FIG 2B

FIG 2C



EDG3 over-expression

Ca-response (fluorescence change counts)

EDG3 + vector    EDG3 + Gq    EDG3 + G16    EDG3 + Gqi5

FIG 2D

FIG 2E



Logarithmic spectrosc...

Ca-response (fluorescence change counts)

12000 10000 8000 6000 4000 2000 0

EDG6 + vector

EDG6 + Gq

EDG6 + G16

EDG6 + Gqi5

FIG 2F

FIG 3

**FIG 4**



Human EDG8 tissue expression

5,5 kb
2,4 kb
ßActin

- brain
- heart
- skeletal muscle
- colon (no mucosa)
- thymus
- spleen
- kidney
- liver
- small intestine
- placenta
- lung
- peripheral blood leukocytes

FIG 5A



522 bp

Pos. control
neg. control
HUVECS
HCAEC
HMVEC-L
HPAEC

FIG 5B

522 bp ➡

EDG- 1 2 3 4 5 6 7 8 H₂O

Fig. 6A
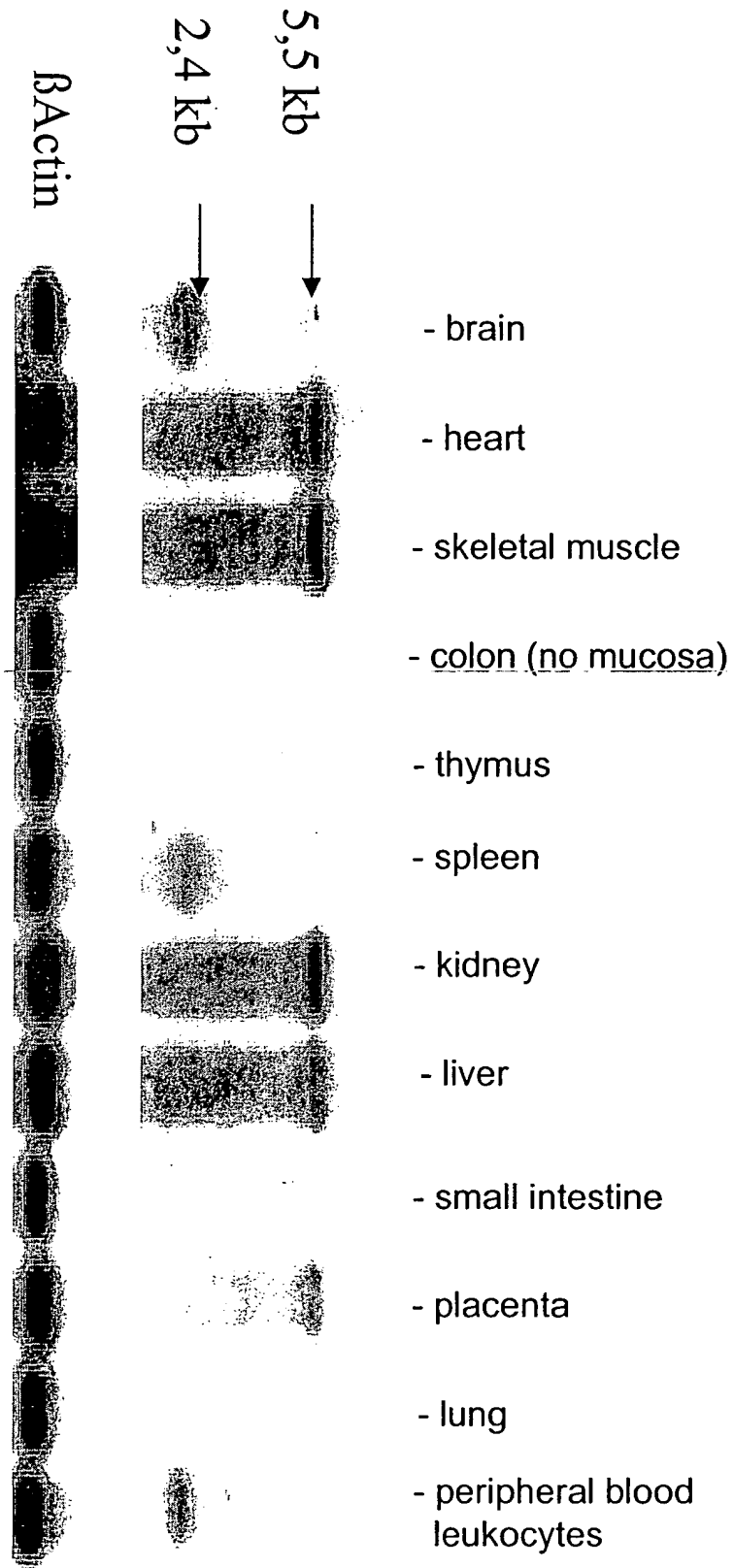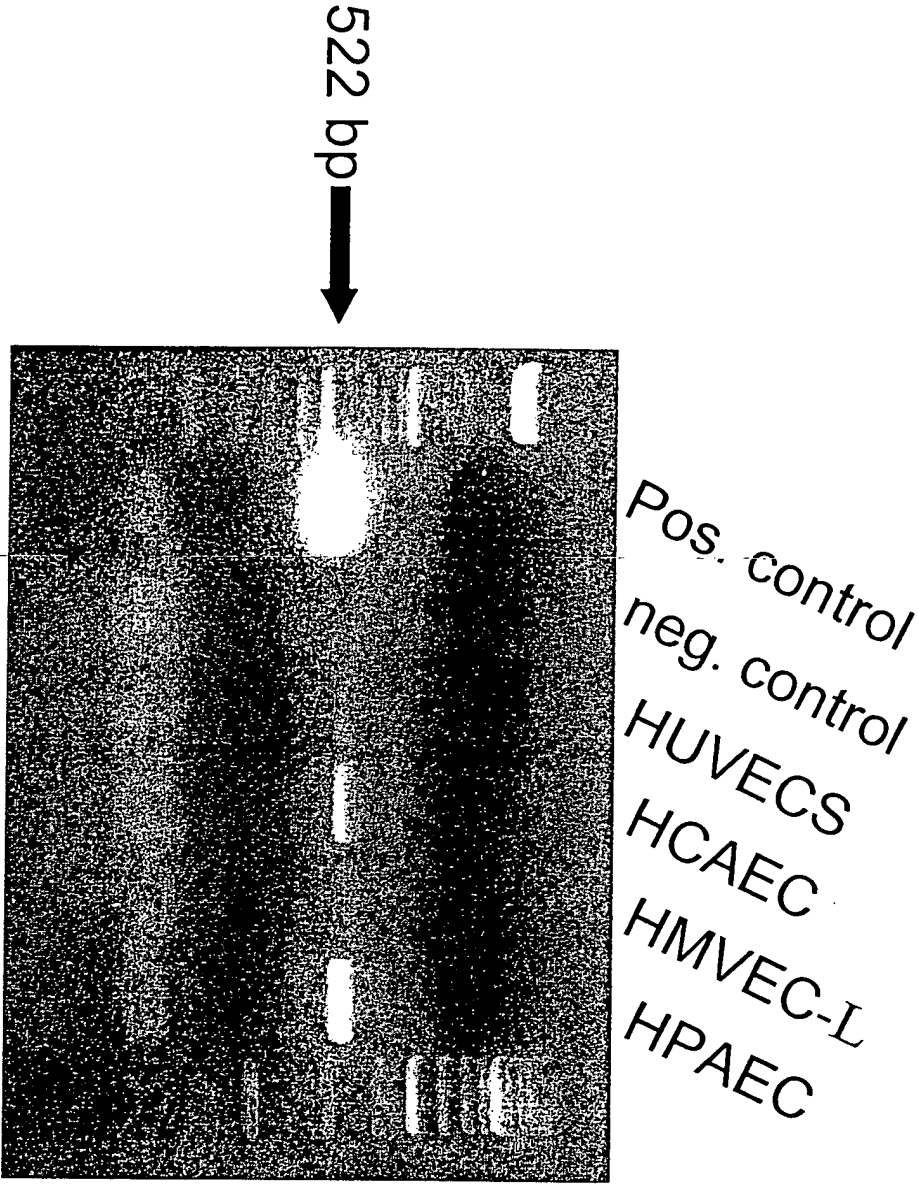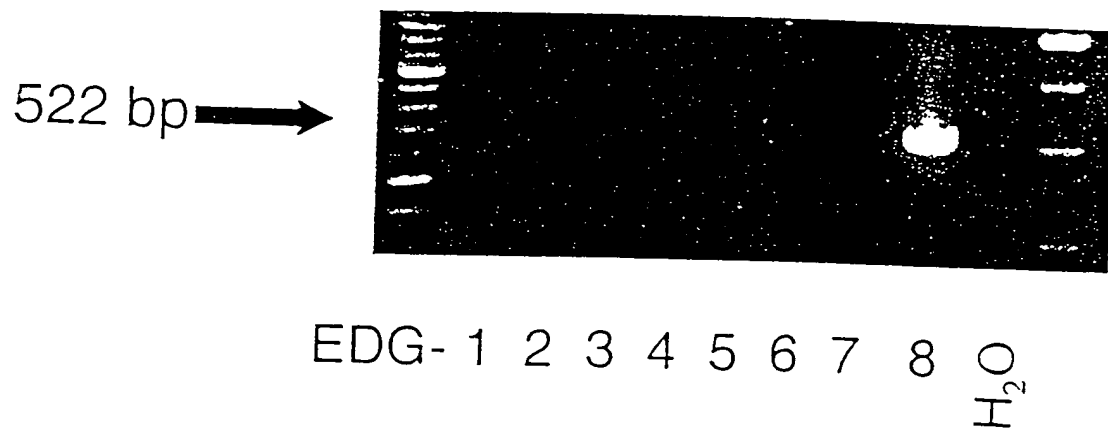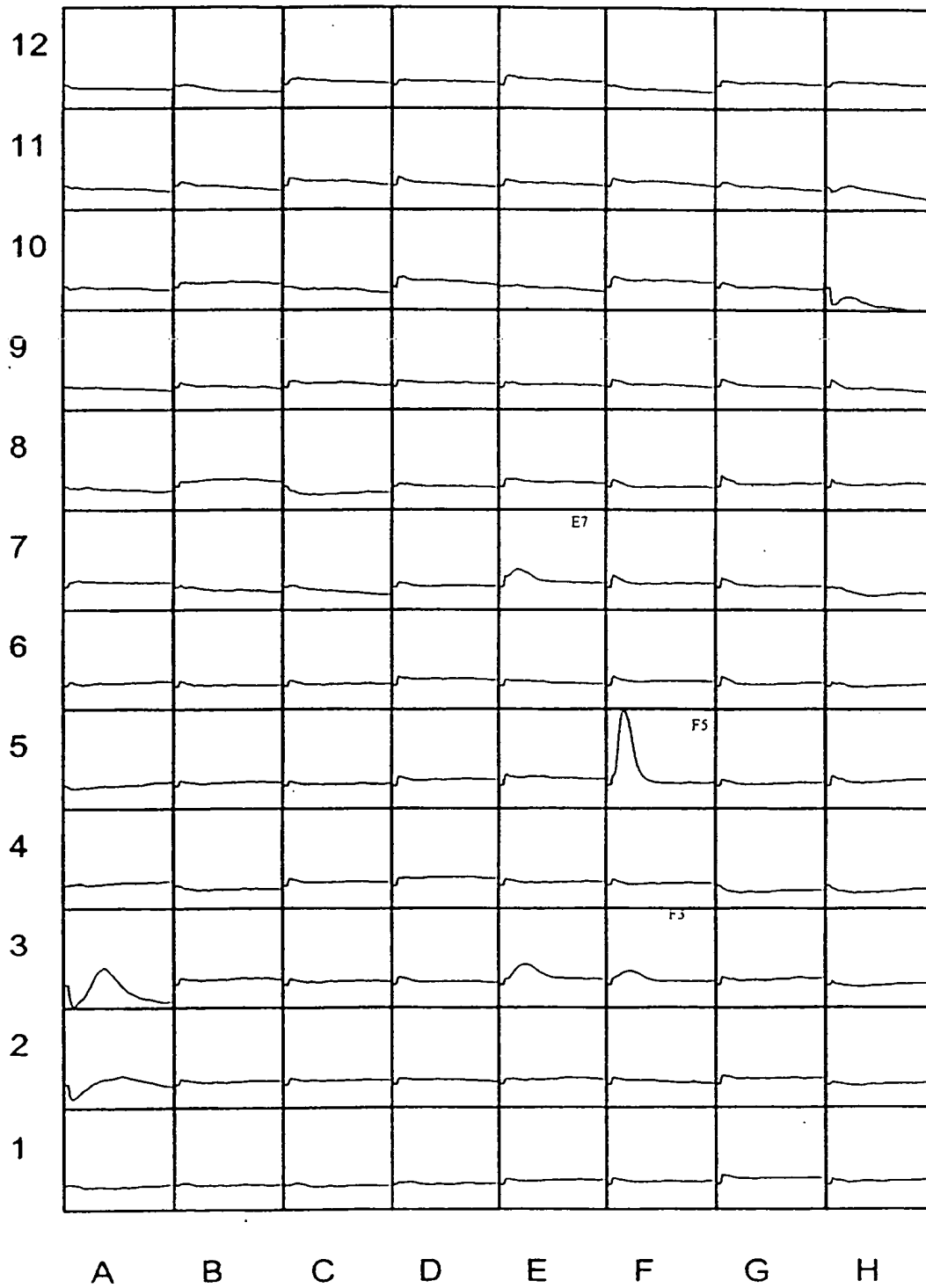
qi5 background

Fig. 6B

rEDG8
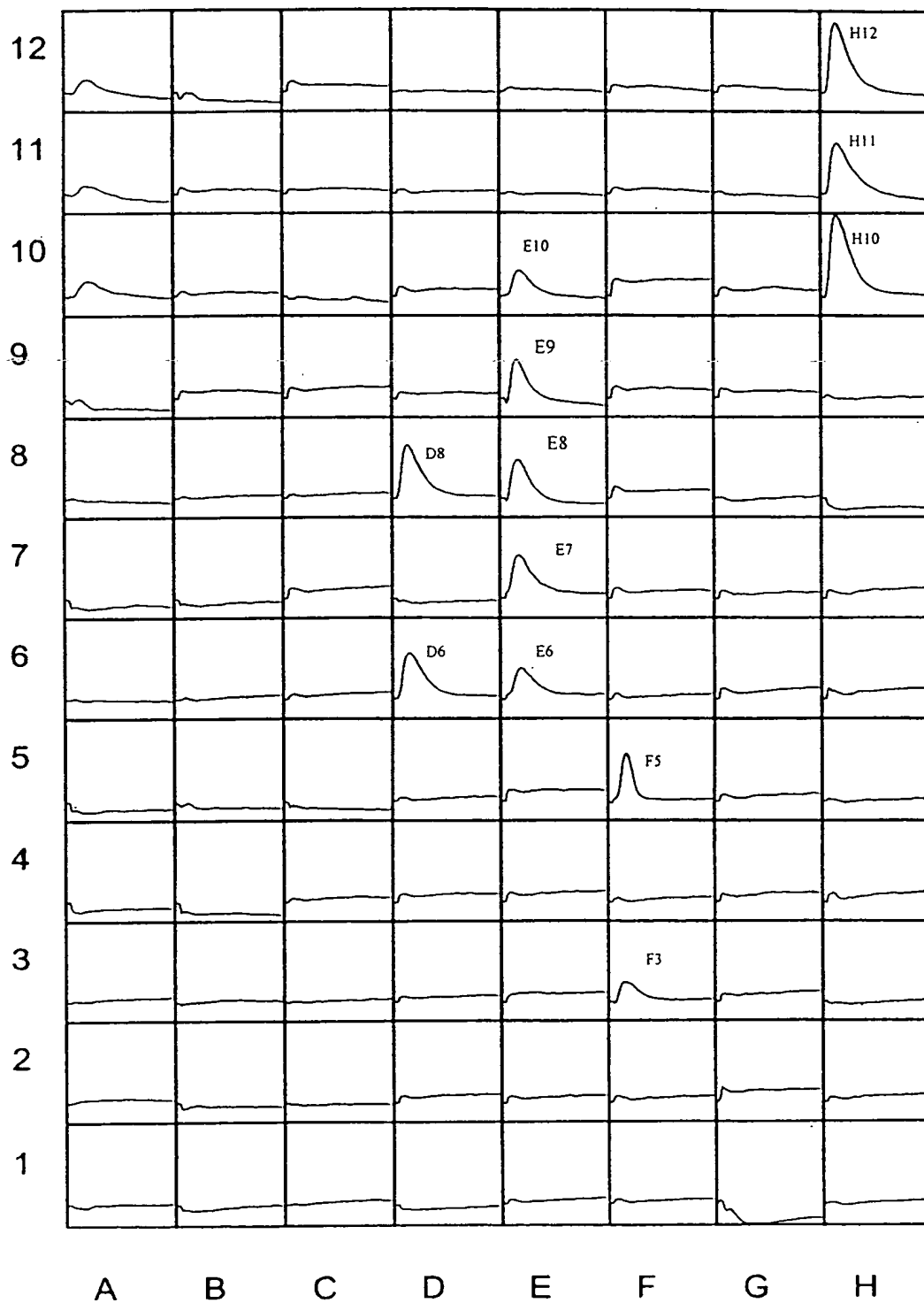
Fig.6C

## Fluorescence Change counts

| Wells | Lipid | background | rEDG8 | stand. response |
|---|---|---|---|---|
| H10-H12 | 1μM S1P | 0 | 5196 | 5196 |
| F5 | 1μM LPA | 5893 | 4327 | -1566 |
| F3 | 1μM cPAF | 1017 | 1570 | 553 |
| E10 | 1μM EPA PAF | 0 | 1354 | 1354 |
| E9 | 1μM AA PAF | 0 | 3121 | 3121 |
| E8 | 1μM Enantio PAF | 0 | 3883 | 3883 |
| E7 | 1μM paf C18:1 | 1256 | 3765 | 2509 |
| E6 | 1μM Lyso PAF | 0 | 2421 | 2421 |
| D8 | 1μM dhS1P | 0 | 5144 | 5144 |
| D6 | 1μM S1P | 0 | 3672 | 3672 |

Fig. 7A

## qi5 background in HEK

Fig. 7B
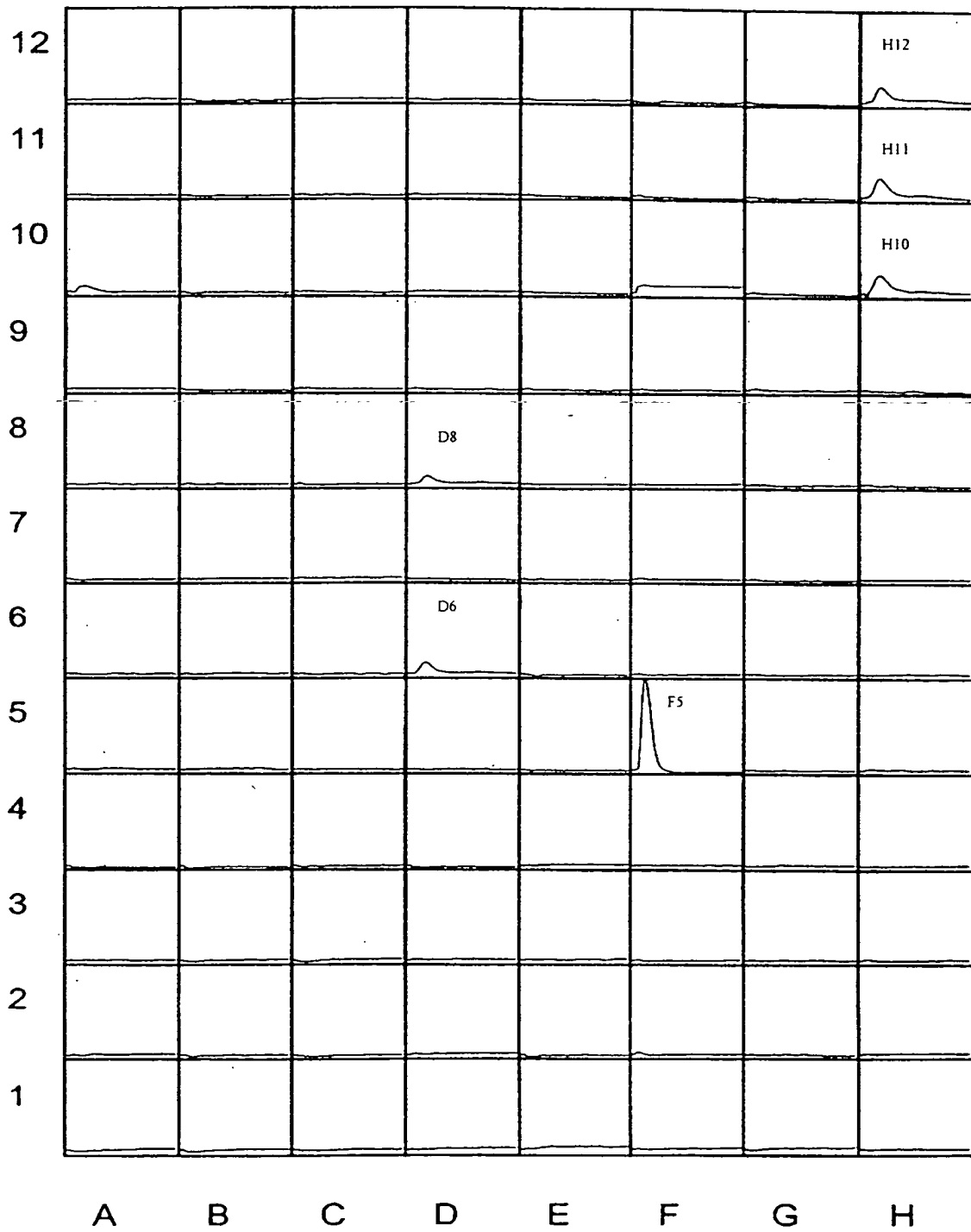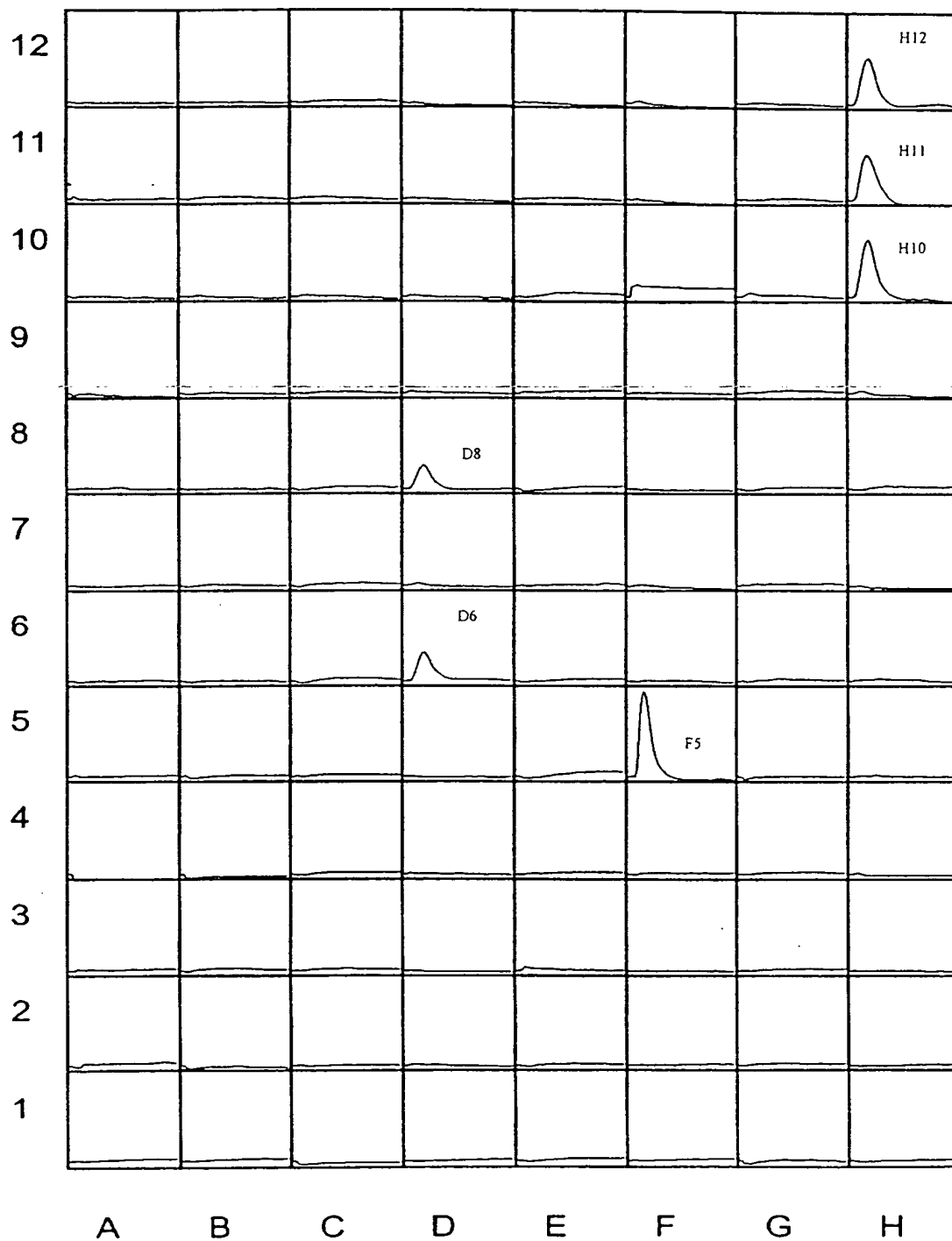
## hEDG8

Fluorescence change counts

| Wells | Lipid | background | hEDG8 | stand. response |
|---|---|---|---|---|
| H10-H12 | 1µM S1P | 3696 | 9493 | 5797 |
| F5 | 1µM LPA | 18004 | 16333 | -1671 |
| D8 | 1µM dhS1P | 1683 | 4522 | 2839 |
| D6 | 1µM S1P | 2273 | 5605 | 3332 |

Fig. 7c

FIG 8
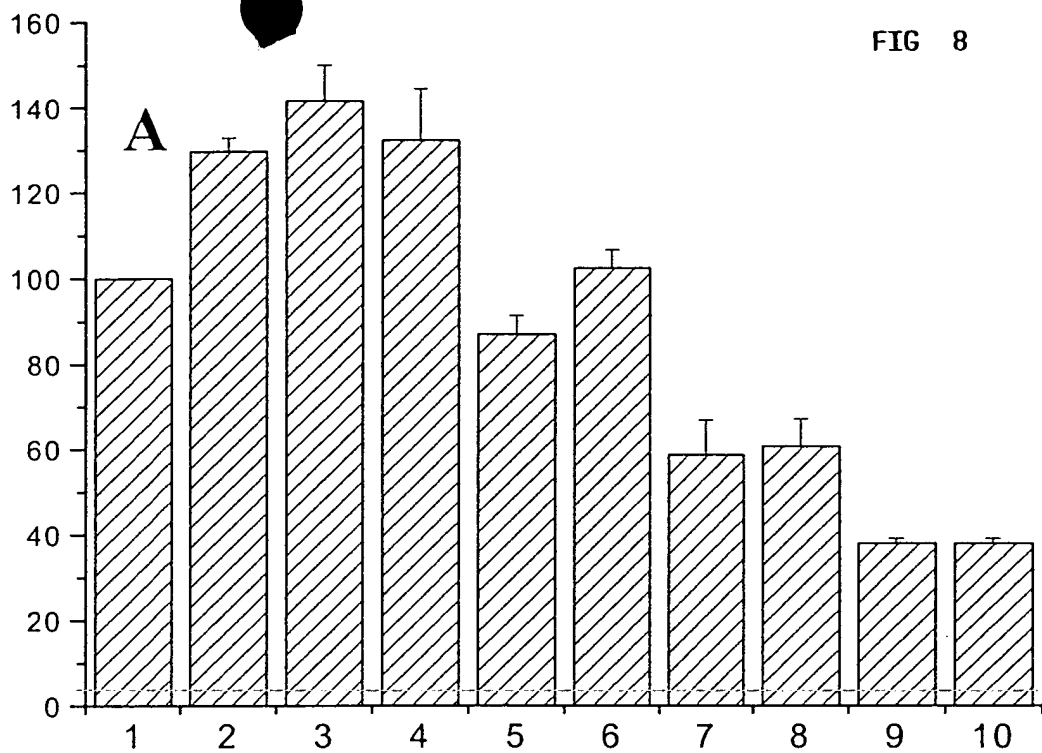


1)S1P 2)Leukotrien B4 3)2 DHLA PAF 4)$C_2$Dihydroceramide
5)15(S)-HEDE 6)PAF C16 7)16,16-Dimethyl $PGE_2$
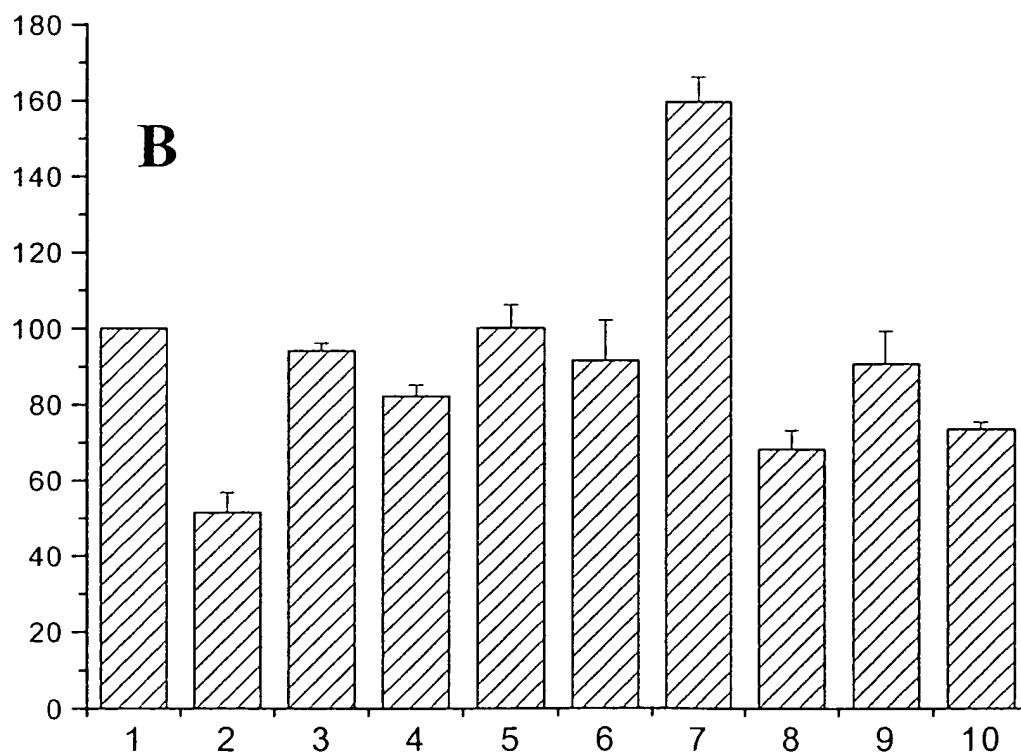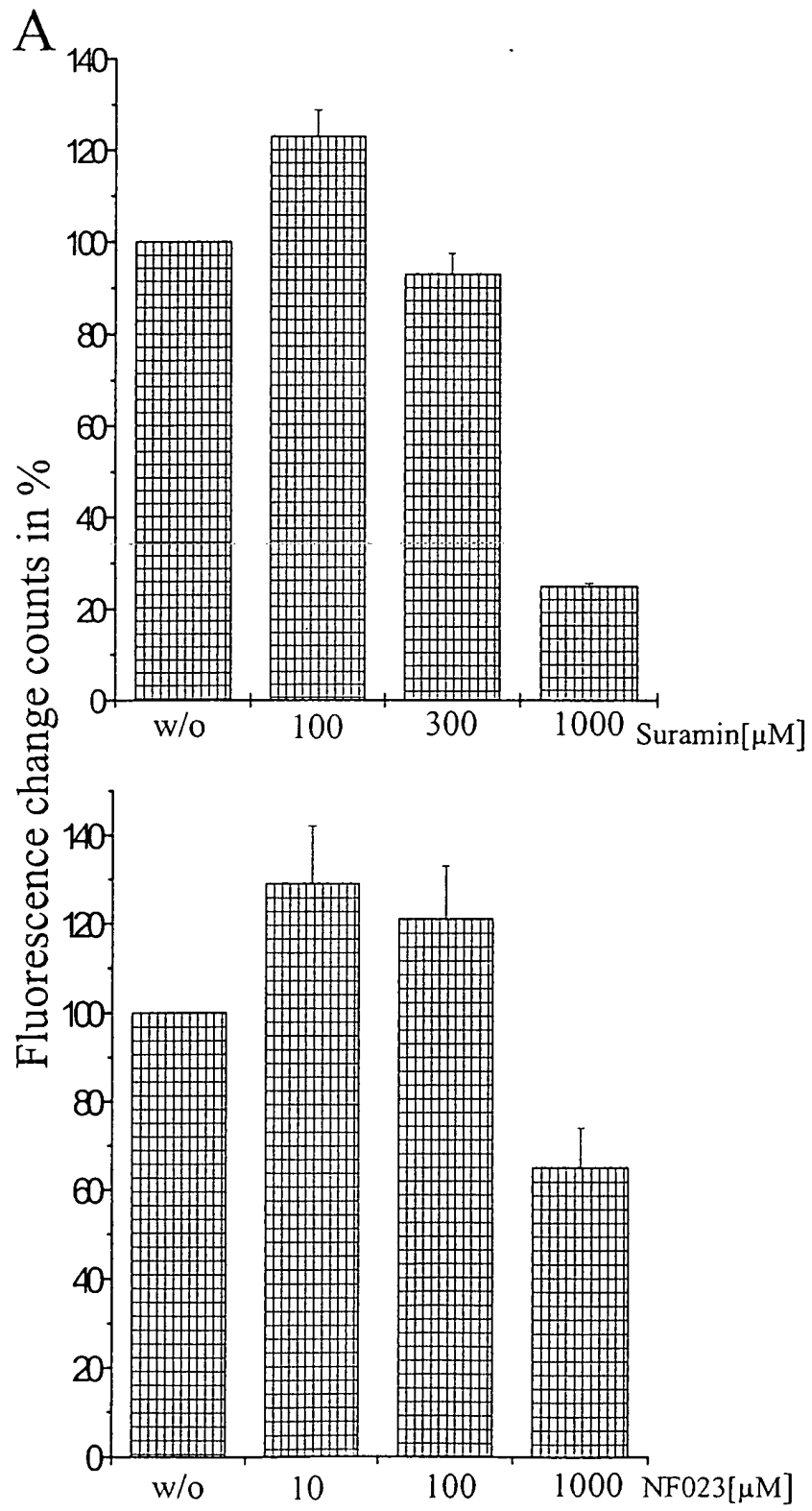8)12(R)-HETE 9)8-Epi-$PGF_{2\alpha}$ 10)Leukotoxin A

% S1P response

FIG 9A

FIG 9B